UK Journal of Pharmaceutical and Biosciences
Available at www.ukjpb.com
ISSN: 2347-9442

Anti- Inflammatory and Diuretic Activities of Ethanolic Extracts Of *Smilax anceps* Wild

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Article Information
Received 5 January 2018
Received in revised form 10 Sept 2018
Accepted 11 Sept 2018

Keywords:
Anti-inflammation,
Diuretic,
Smilax anceps,
Ethanolic extracts

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Abstract

The anti- inflammatory and diuretic activities of ethanolic extracts of *Smilax anceps* Wild was studied. Leaves of the plant were collected from the premises of Michael Okpara University of Agriculture, Umudike Umuahia. The leaves were sun-dried for eight days, ground and macerated in 300ml of ethanol and left to stand for 24 hours. The solution was thereafter filtered using No 1 Whatman filter paper and allowed to evaporate to dryness, to obtain the extract used for the study. Twenty adult rats were used for this investigation. They were allowed to acclimatize to the environment for two weeks. The extracts were administered to the rats at doses of 125, 250, 500, 1000 and 2000 mg/kg body weight. The administration was by intra-peritoneal during the diuretic test and orally during anti-inflammatory test. For the anti-inflammatory test, aspirin was used as positive control and normal saline as negative control. In the diuretic test, four standard drugs (Furosemide, Hydrochlorothiazide, Spironolacetone and Acetazolamide) and normal saline served as control. The results obtained indicate that the plant extracts significantly ($P<0.05$) inhibited the inflammation of the hind paws of the rats showing that the extracts had an anti-inflammatory effect. The rate of inhibition increased with increase in the concentration of the extracts. The plant extracts also significantly ($P<0.05$) increased the volume of urine produced by the rats, indicating that the leaves had diuretic effect. Higher volumes of urine were produced as the concentration of the extracts increased. The results obtained showed that ethanolic leaf extracts of *Smilax anceps* have potential anti-inflammatory and diuretic properties, hence could be utilized in the treatment of inflammation and diuretic problems.

1 Introduction

Medicinal plants for many years are known to be source of unknown chemical substances with potential therapeutic effects[1]. World Health Organization estimated that over 75% of the world's populations still rely on plant derived medicines usually obtained from traditional healers for basic health care needs[2]. Osuagwu *et al*, (2010)[3] also reported that in Nigeria, a developing country, most people still depend on herbs as a cheap readily available and alternative source of drugs. There have been the use of different plant extracts and formulations to alleviate and treat diseases for many centuries[4]. Technological developed countries such as United States of America and Japan have about 60% of the population still rely on medicinal plants for a therapeutic purpose for certain diseases[5]

Drugs which are used to increase urinary output and electrolytes excretion are known as diuretics[6]. These drugs mostly act on different parts of nephrons and increase urine volume[7]. These drugs are known to relieve pulmonary congestion and peripheral oedema thereby decreasing cardiac work load, oxygen demand and plasma volume leading to decrease in blood pressure[1]. The use of plant based materials as diuretics has been reported. Plant such as *Allium sativa*, *Salvadora perica*, *Mentia viridis*, have been observed to have diuretic effects[8,9].

According to Kumar et at, 2013[10], inflammation is the protective response to tissue injury which is caused by physical trauma, noxious chemicals or microbiological agents. Inflammation process involves a complex a complex array of enzyme activation, mediators, release extravasation cell migration, tissue breakdown and repairs[11].. Inflammation may be acute, which is associated with increased vascular permeability, infiltration and emigration of leucocytes or chronic inflammation which is associated with infiltration of mono-nuclear immune cells, macrophages, monocytes, neutrophils fibroblast activation and proliferation[10]. The symptoms of inflammation include pains, swelling and loss of function which may be caused by inflammatory agents[12].

Available reports have indicated that some plants have anti-inflammatory abilities[13-18]. The report of Durmowicz and Stenmark, 1999[19] indicated that these medicinal plants contain enormous molecules that act synergistically on targeted elements of the complex cellar pathway.

Smilax anceps is a vigorous scrambling vine or shrub which belongs to the Smilaceae family[20]. It is a spiny climbing lianas up to 10 – 12 m long. Stem is glabrous, brownish usually bearing two simple tendrils at leaf base. Thorns are irregularly scattered all long the stem curved squart, 1- 4 mm long. Leaves are alternate, glabrous, ovate or elliptical lanceolate, 5 – 12 cm long and 2 – 5 cm across with rounded or sub cordate base. Petioles are glabrous, 0 – 25 cm long[20].

The parts of the plant have been reported to be utilized in treating diseases. The roots are diuretic, purgative, diaphoretic. They are also used in treating syphilis, gastralgia and albuminuria in pregnant women[20]. The stems are used in treating rheumatism, paralysis, teething in young children and scabies. The leaves are used in curing rheumatism, paralysis, scabies,opthalmia and snake bites[20]. .*S. anceps* have also been shown to be used in treatment of female infertility, drepanocytosis, boils, and malaria[21]. Furthermore, the leaves of S. anceps have beeb implicated in the treatment of skin spots[22]. The therapeutic properties of S. anceps are due to its phytochemical constituents. It is reported to contain alkaloids and saponin[23].

The objective of this research was to investigate the anti-inflammatory and diuretic activities of the ethanolic leaves extracts of *smilax anceps*, in view of utilizing the leaves extracts as alternative source of drugs for the treatment of inflammation and diuretic related ailments.

2 Materials and methods

2.1 Plant samples

The leaves of *Smilax anceps* were collected from the premises of Michael Okpara University of Agriculture, Umudike, Umuahia Abia State. The leaves were identified by the taxonomic unit of the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.

2.2 Preparation of plant extracts

The leaves were sundried for eight days and ground into powder using Thomas Willing Milling Machine. 50 g of powdered leaves were macerated in 300 ml of ethanol and left to stand for 24 hours. The solution was filtered using No 1 Whatman Filter Paper and filtrate was exposed to the atmosphere for evaporation to obtain the need extract.

2.3 Animal stock

Twenty Albino rats of both sexes aged between 4 – 8 weeks and weighing between 97-206 g were used for the study. The animals were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. They were kept in well aerated laboratory cages in the University Animal House and were allowed to acclimatize with the environment for a period of two weeks before treatment commenced. The animals were also given pellet food drinking water *ad libitum*.

2.4 Acute toxicity study

The method of Lorke (1983)[24] was used to determine the acute toxicity (LD 50) of the extracts. 20 rats weighed and divided into four groups were used for the actor toxicity study. Four different concentrations were made from the stock solution of the extracts to mg/ml, 500mg/ml, 1000mg/ml and 2000mg/ml respectively. The weight of each animal determined the amount of the extract given to the animals. These were given intra-peritoneally and after 72 hours percentage mortality of the animals were known.

2.5 Anti-inflammatory activity

The anti-inflammatory activity of the extracts of *Smilax anceps* was determined using the method of Okoli and Akah, (2000)[25].

Fourteen (14) adult albino rats of both sexes and average weight of 149.5g were used for the study. The animals were grouped into seven (A-G) consisting of two animals per group and placed in cages. The animals were allowed to feed prior to the experiment but were deprived access to both feed and drinking water during the experiment. Treatments were administered orally.

The GroupA animals were administered with 50 mg of aspirin and served as positive control. Group B animals neither received aspirin or plant extract but normal saline thus served as negative control. Animals in groups C to G were given plant extracts 125 mg/kg, 250 mg/kg, 500 mg/kg, 1000 mg/kg, 2000 mg/kg respectively. The animals were left for 10 minutes after which 0.1 ml fresh egg albumin was injected into the sub planter of the right hind paw of each rat. The diameter of the hind paw

was measured at 30 minutes interval for 2 ½ hours, using a vernier caliper.

2.6 Diuretic activity

The assessment of diuretic activity of the leaves of *smilax anceps* was carried out using the method of lipschiz*et al*, (1993)[26].

The albino rats weighing about 100-150 g were deprived of food and water for 10 hours prior to treatment. The experimental animals were divided into ten groups of two animals each. The animals in group 1 received normal saline which served as control. Group 2 animals were administered with furosemide, group 3 animals received Thiazide, group 4 animals were injected with spironolactone and group 5 animals were given Acetazolamide. Group 6 to 10 animals were the test group and were administered different doses of the plant extracts at the dose of 125 mg/kg, 250 mg/kg, 500 mg/kg, 1000 mg/kg and 2000 mg/kg. The treatment was carried out intra-peritoneally

After the treatment, the animals were placed separately in aerated cages. Syringes were used to collect the urine produced after 24 hours. The animals were not given water nor food during the test and were closely monitored. The total volume of urine and concentrations of sodium ion, potassium ion, chloride ion and bicarbonate in the urine were determined.

2.7 Statistical analysis

The data obtained were expressed as means standard error for the animals in each group. Data were analyzed using the one-way analysis of variance (ANOVA) in all cases and statistical significance was established at values ($P < 0.05$).

3 Results

The results of the anti-inflammatory and diuretic activities of ethanolic extracts of *Smilax anceps* leaves are summarized in tables 1 to 3.

Extracts of the leaves of *S. anceps* significantly ($P < 0.05$) affected the inflammation of the paws of the test rats. Generally, the leaf extracts reduced the rate of the inflammation of the paws of the rats (Table 1).

Table 1: The effect ethanolic extracts of *Smilax anceps* leaves on hind paw (Edema) in diameter (mm)

Doses	0 mins	30 mins	40 mins	120 mins	160 mins	150 mins
Positive control	16.0 ± 1.41^{cd}	13.00 ± 2.63^{ab}	10.0 ± 1.00^{a}	11.3 ± 0.71^{a}	12.00 ± 1.41^{bc}	11.5 ± 2.12^{b}
Negative control	10.5 ± 3.54^{a}	9.5 ± 3.54^{a}	11.5 ± 3.54^{b}	12.5 ± 3.54^{b}	11.5 ± 3.54^{b}	11.5 ± 3.34^{b}
125 mg/kg	13.0 ± 4.24^{b}	10.5 ± 2.12^{a}	14.5 ± 2.12^{bc}	12.00 ± 1.41^{ab}	12.00 ± 1.41^{bc}	12.0 ± 1.41^{b}
250 mg/kg	10.0 ± 1.00^{a}	13.5 ± 3.54^{b}	12.00 ± 4.24^{b}	12.5 ± 3.54^{b}	13.5 ± 2.12^{c}	13.5 ± 0.71^{c}
500 mg/kg	13.5 ± 2.83^{b}	9.5 ± 1.71^{a}	13.10 ± 1.14^{c}	13.5 ± 3.54^{c}	12.5 ± 4.95^{c}	10.5 ± 2.2^{ab}
1000 mg/kg	13.5 ± 7.78^{b}	14.5 ± 5.66^{b}	13.5 ± 2.12^{c}	11.5 ± 2.12^{a}	12.5 ± 3.34^{c}	9.5 ± 0.71^{a}
2000 mg/kg	15.00 ± 2.83^{c}	16.1 ± 1.41^{c}	$16.0 \pm 1\ 41^{d}$	13.00 ± 1.41^{bc}	10.5 ± 1.70^{a}	9.5± 0.75a
LSD ($P < 0.05$)	1.15	1.06	1.50	1.45	1.38	1.05

Values are presented as means of three replicates. Means of different superscripts are significantly different ($P< 0.05$)

The average inhibition of the edema by the positive control (aspirin) at 0 minute was 16 ± 1.4 and 11.5 ± 2.12 after 150 minutes, while the average inflammatory inhibition of the highest concentrations of the extracts (2000 mg/kg) was 15.0 ± 2.83 at 0 minutes and 9.5 ± 0.71 after 150 minutes. On the other hands, the test concentration of the extracts (125 mg/kg) had the average inflammatory inhibition 13.0 ± 4.24 at 0 minutes and 12.0 ± 1.41 at 150 minutes. This indicates that as the concentration of the extracts increased, there was significant reduction in the diameter of the edema.

The extracts of the leaves of *S. anceps* significantly ($P < 0.05$) affected the volume of urine produced by the rats. The leaf extracts generally increased the volume of urine produced by the rats (Table 2). The negative control (saline) produced 0.95 ± 0.35 ml of urine and the positive controls (Furosemide, Hydrochlorothiazide, Spironolactone and Acetazolamide) produced 0.30 ± 0.28, 0.20 ± 0.12, 0.20 ± 0.14, 0.30 ± 0.14 ml respectively, while the highest concentration of the extracts (20000 mg/kg) produced 0.96 ± 0.70 ml of urine and the least concentration 125 mg/kg produced 0.40 ± 0.28 ml of urine. The result obtained also indicates that as the concentration of the extract increased the volume of the urine produced also increased.

Extracts of the leaves of *S. anceps* generally, significantly ($P < 0.05$) affected the excretion of electrolytes by the rats (Table 3). The excretion of sodium, chloride, potassium and bicarbonate ions due to treatment with the negative control (normal saline) was 75.6 ± 1.06, 72.9 ± 0.76, 16.9 ± 0.78 and 26.6 ± 0.35 respectively, while those of the highest concentration of the extracts were 145.3 ± 0.5, 142.9 ± 0.9, 23.8 ± 0.35 and 29.0 ± 0.21 respectively. The result obtained shows that electrolytes

excretion increased as the concentration of the extract given increased (Table 3).

Table 2: The effect of the ethanolic leaf extracts *S. anceps* on volume of urine (ml) produced by the test animals

Dose	Volume of urine (ml)
Normal saline	0.95 ± 0.35
Furosemide	0.30 ± 0.28
Hydrochlorothiazide	0.20 ± 0.10
Spironolacetone	0.20 ± 0.14
Acetazolamide	0.30 ± 0.14
125 mg/kg	0.40 ± 0.28
250 mg/kg	0.60 ± 0.40
500 mg/kg	0.80 ± 0.50
1000 mg/kg	0.80 ± 0.57
2000 mg/kg	0.98 ± 0.70

Values are presented as means of three replicates

4 Discussions

The leaves of *Smilax anceps* exhibited significant anti-inflammatory and diuretic activities. The ethanolic leaf extracts significantly ($P < 0.05$) reduced the rate of inflammation of the paws (edema) of the rats, when compared with the positive control (aspirin). This indicates that the leaf extracts had anti-inflammatory activity. The anti-inflammatory activity of plants parts has also been reported by researchers[18,27,28]. The anti-inflammatory activity of these medicinal plants might be due to the presence of phytochemicals such as alkaloids, glycosides, flavonoids, phenolic compounds, steroids, saponins etc they contain[15,27,29,30]. *S. anceps* leaves are known to contain alkaloids, flavonoids, saponins and tannins[23,31]

The ability of the extracts *S. anceps* to inhibit inflammation of the rat paws increased with increase in the concentration of the extracts. Thus indicating that the effect was concentration dependent. Similar observation was made in other plants[18,32].

The extracts of the leaves of *S. anceps* significantly ($P < 0.05$) increased the volume of urine produced by the rats. This showed that the extracts had diuretic effect. Diuretic activity of plant extracts has also been reported[33.34,35,36].

Table 3: The effect of the ethanolic leaf extract of *S. anceps* on electrolytes excreted by the test animals

Dose	Na^+	Cl^-	K^+	HCO_3
Normal saline	75.6 ± 1.06^{b}	72.6 ± 1.06^{b}	16.9 ± 0.92^{b}	26.6 ± 0.35^{c}
Furosemide	157.5 ± 0.99^{ed}	154.5 ± 0.28^{d}	26.2 ± 0.85^{e}	34.2 ± 0.92^{c}
Hydrochlorothiazide	168.3 ± 1.56^{e}	167.9 ± 3.67^{e}	30.30 ± 1.84^{f}	35.0± 0.72^{e}
Spironolactone	154.6 ± 2.4^{ef}	151.4 ± 1.70^{d}	15.7 ± 0.55^{a}	29.7 ± 1.10^{d}
Acetazolamide	80.1 ± 1.21^{b}	86.5 ± 1.50^{bc}	27.4 ± 0.64^{c}	71.6 ± 3.10^{a}
125 mg/kg	53.5 ± 1.56^{a}	50.2 ± 0.14^{a}	12.0 ± 0.92^{a}	21.6 ± 0.35^{a}
250 mg/kg	77.4 ± 1.34^{b}	70.1 ± 1.90^{b}	14.0 ± 1.13^{ab}	23.0 ± 0.70^{b}
500 mg/kg	110.1 ± 1.11^{c}	109.0 ± 3.75^{c}	18.2 ± 0.72^{c}	33.1 ± 0.78^{e}
1000 mg/kg	110.1 ± 1.11^{c}	110.5 ± 3.50^{c}	19.5 ± 1.12^{c}	30.5 ± 0.90ef
2000 mg/kg	145.3 ± 1.50^{e}	142.9 ± 1.99^{d}	23.8 ± 0.35^{d}	29.0 ± 0.40^{c}
LSD(P<0.05)	0.01	11.26	0.17	0.23

Values are presented as means of standard deviation. Means of different superscripts are significantly different (p< 0.05)

The diuretic activity of plant materials might be due to their phytochemical content. Asif*et al*, 2013[34] and Hallu and Engida-work, 2014[35] reported that the presence of phenolic compound, tannins, saponins, flavonoids, terpenoids, steroids, alkaloids and other secondary metabolites were responsible for the diuretic activity of *Ajuga remota* and *Trianthema portulacastrum*. The phytochemical screening of *S. anceps* revealed the presence of alkaloids, flavonoids saponins and tannins[23,31]. The volume of urine produced by the rats increased as the concentration of *S. anceps* extracts increased. Similar observation was also reported by other researchers[15,18,37].

The excretion of electrolytes by the rat samples was significantly ($P < 0.05$) increased by treatment with leaf extracts of *S. anceps*. The reports of increased excretion of electrolytes due to treatment with plant extracts have been made by some workers[33,34,36,38]. The electrolytes excrete due to treatment with *S. anceps* leaf extracts increased with increased in the concentration of the extracts. This implies that the excretion of

electrolytes was concentration dependent. Some researchers also had made similar observation[10,18,33].

From the results obtained due to treatment of the rats with leaf extracts of *Smilax anceps*, it could be concluded that the extracts have anti-inflammatory and diuretic properties hence could be utilized in the treatment of these health conditions.

5 Conclusion

The research considered the anti-inflammatory and diuretic activities of the ethanolic extracts of the leaves of *Smilax anceps.* From the results obtained it could be concluded that the ethanolic extracts of the leaves of *S. anceps* had anti-inflammatory and diuretic properties. Thus indicating that they could be utilized in the treatment of these health conditions.

The concentration of the extracts determine the rate of activity of the extracts. The rate of activity increased with the concentration of the extracts.

6 Conflict of Interest

None.

7 Authors contributions

OGGE supervised the research and wrote the manuscript, OAN and UNA carried out the research and gathered the data, while UFO analyzed the data and proof read the write up.

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